

99* Roscovitine: a novel corrector for the functional rescue of F508del-CFTR protein

C. Norez¹, C. Vandebrouck¹, L. Meijer², F. Becq¹. ¹IPBC, Université de Poitiers, CNRS, Poitiers, France; ²Station Biologique, CNRS, Roscoff, France

The most common mutation in Cystic Fibrosis, F508del, causes defects in trafficking, channel gating and endocytosis of the CFTR (CF Transmembrane conductance Regulator) protein. We have previously developed an automatic assay to identify agents, termed correctors, directed at repairing these defects. This study shows the identification from our screening of a novel corrector, roscovitine, able to restore the membrane localization and functionality of F508del-CFTR protein on the human airway epithelial CF cell line JME/CF15.

We found that roscovitine corrected F508del-CFTR with an EC₅₀ of 56 μM. Moreover, biochemical analysis and immunofluorescence imaging confirmed the restoration of a mature F508del-CFTR at the cell surface. To pinpoint the molecular mechanism of roscovitine, we realized competition studies between roscovitine and inhibitors of the endoplasmic reticulum quality control (ERQC). No potentiation of roscovitine correction occurred after co-treatment by roscovitine and the degradation inhibitor MG132. Only a small potentiation was obtained after co-treatment by roscovitine and thapsigargin which inhibits interaction between CFTR and calnexin, a Ca²⁺-dependent ER chaperone. Additional studies showed that roscovitine stimulates an intracellular Ca²⁺ increase, consecutive of the ER emptying. Moreover a Ca²⁺-independent inhibition of proteasome activity (~50%) in CF15 cells was observed after a roscovitine treatment.

We conclude from the present study that roscovitine restores the abnormal trafficking of F508del-CFTR via a Ca²⁺-dependent and a Ca²⁺-independent mechanisms of action allowing disturbance of the ability of the ERQC to interact and to degrade F508del-CFTR proteins.

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101* Relationship between pulmonary exacerbations and lung function decline in a six month trial of denufosal

F.J. Accurso¹, T.A. Durham², A.E. Schaberg². ¹The Children's Hospital, Denver, CO, USA.; ²Inspire Pharmaceuticals, Durham, NC, USA

The relationship between pulmonary exacerbations (PEX) and lung function decline in cystic fibrosis (CF) is not completely understood. The purpose of this research was to describe changes in lung function by occurrence of PEX among CF patients participating in a randomized, double-blind, placebo-controlled trial of denufosal. A total of 352 patients ≥5 years of age in the US and Canada with a screening FEV1% predicted ≥75% were randomized to receive either denufosal or placebo three times per day for 24 weeks. Lung function was measured using central spirometry at 6 study visits. PEX, defined as reporting at least 4 out of 12 Fuchs' criteria regardless of treatment, were tracked during the study. Differences in the change from baseline (CFB) FEV1 with respect to treatment and exacerbation were tested via analysis of covariance (ANCOVA) models. Overall, the adjusted mean CFB FEV1 was -34 mL for patients with PEX (n=80) vs. +43 mL for patients free from PEX (n=272; p=0.006). Among patients who experienced a PEX, those treated with denufosal (n=47) had an adjusted mean CFB of +2 mL in FEV1 vs. -99 mL for those treated with placebo (n=33; p=0.06). PEX in both treatment groups were similar relative to the demographics of patients, duration of PEX, time to PEX, and treatment with IV antibiotics. These data suggest loss in lung function is greater in patients who experience PEX than in patients PEX-free. In addition, denufosal may attenuate the effects of PEX on lung function.

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100* Effects of denufosal on sinusitis-related complaints in a phase 3 trial in cystic fibrosis patients

A.R. Mospan¹, T.A. Durham¹, A.E. Schaberg¹, F.J. Accurso². ¹Inspire Pharmaceuticals, Inc., Durham, NC, USA; ²The Children's Hospital, Denver, CO, USA

Sinusitis is common in patients with CF. Current treatments do not target defective ion transport, which may contribute to the pathogenesis of chronic sinus disease in CF. Denufosal is a novel inhaled P2Y₂ receptor agonist designed to treat the ion channel defect in CF lung disease by stimulating an alternative chloride channel (calcium-activated chloride channel), water and mucin secretion and by increasing cilia beat frequency on airway epithelia to enhance mucociliary clearance. TIGER-1 (Study 08-108) was a 6-month placebo-controlled, double-blind, multicenter Phase 3 study comparing the effects of denufosal and placebo (normal saline) administered three-times-daily via jet nebulizer on lung function and other parameters in 352 patients ≥5 years of age with CF. In addition to a significant benefit in FEV₁ at the primary efficacy endpoint, there were significantly fewer reports of adverse events (AEs) typically associated with sinusitis. Among the most commonly reported AEs (≥ 10% in any group) during the 6-month placebo-controlled study, significantly fewer events were reported by patients who administered denufosal compared to placebo with respect to sinusitis (10% vs 18%; p=0.030), headache (11% vs 25%; p=0.001) and rhinorrhea (10% vs 18%; p=0.046). Post-nasal drip AEs were also diminished for denufosal-treated patients (2% vs 5%; p=0.085). Sinus congestion occurred more frequently for denufosal-treated patients (7% vs 3%; p=0.088). These data provide preliminary evidence that denufosal may have the potential to reduce the occurrence of sinus-related complaints in CF patients, presumably due to activity on the sinus and nasal epithelial surface.

102* The novel long acting ENaC blocker P643 has therapeutic effects in chronic cystic fibrosis-like lung disease in mice

Z. Zhou¹, M. Harm¹, D.A. Treis¹, S.C. Schubert¹, J. Schatterny¹, S. Hirtz¹, J. Duerr¹, A.J. Hirsh², M. Ross Johnson², R.C. Boucher³, M.A. Mall¹. ¹Ped. Pulmonology and CF Center, Dep. of Pediatrics III, University of Heidelberg, Heidelberg, Germany; ²Parion Sciences Inc., Durham, NC, USA; ³CF Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA

Increased ENaC-mediated airway Na⁺ absorption is a characteristic abnormality in the pathogenesis of cystic fibrosis (CF) lung disease and causes CF-like lung disease in mice. We recently demonstrated that preventive, but not late, ENaC inhibition by its classical blocker amiloride reduced mortality and morbidity of CF-like lung disease in βENaC-transgenic (βENaC-Tg) mice (Zhou Z. *et al*, 2008). We hypothesized that the ineffectiveness of late amiloride therapy was due to (i) the low potency and rapid absorption of amiloride from airway surfaces, or (ii) irreversible lung pathology. To distinguish between these possibilities, we used a novel, highly potent and long acting ENaC blocker, P643 (Parion Sciences), shown to be 35–60-fold more potent and ~5-fold longer acting than amiloride *in vitro*, and tested the therapeutic benefits of P643 on CF-like lung disease in mice. Newborn or 4-week old βENaC-Tg and wild-type mice were treated by intrapulmonary administration of P643 or vehicle alone for 2 weeks. Subsequently, mice were euthanized, bronchoalveolar lavage performed, and lungs processed for histology. We demonstrate that P643 is an effective preventive therapy, and that late intervention with P643 significantly reduced airway mucus obstruction, neutrophilic inflammation and epithelial remodeling in established lung disease in βENaC-Tg mice. These results suggest that long acting ENaC blockers may have therapeutic effects in CF patients with chronic lung disease.

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